New Forests **6**: 391–407, 1992.

Review paper

Use of DNA markers in forest tree improvement research

D. B. NEALE, M. E. DEVEY, K. D. JERMSTAD, M. R. AHUJA, M. C. ALOSI and K. A. MARSHALL

Institute of Forest Genetics, Pacific Southwest Research Station, Box 245, Berkeley, CA 94701, USA

Received 15 December 1990; accepted 3 March 1992

Key words: DNA markers, genetic maps, RFLPs

Application. Development of DNA markers will provide abundant new genetic markers for forest tree improvement research. DNA markers will be most useful for estimating genetic diversity in breeding populations and for germplasm identification. Eventually, high-density maps may be used to identify quantitative trait loci and to practice marker-assisted selection.

Abstract. DNA markers are rapidly being developed for forest trees. The most important markers are restriction fragment length polymorphisms (RFLPs), polymerase chain reaction- (PCR) based markers such as random amplified polymorphic DNA (RAPD), and fingerprinting markers. DNA markers can supplement isozyme markers for monitoring tree improvement activities such as; estimating genetic diversity in breeding populations, germplasm identification, verifying controlled crosses, and estimating seed orchard efficiencies. Because the number of DNA markers is potentially limitless, it should be possible to map individual quantitative trait loci (QTL) by linkage analysis with high-density maps. Finally, if such associations can be found, it may also be possible to design marker-assisted breeding strategies for forest trees.

Introduction

Genetic markers are important tools for forest tree improvement. Isozyme markers have been applied extensively during the past 15 years and have contributed significantly to tree breeding programs (Adams 1981a, 1983; Adams et al. 1988; Cheliak et al. 1987; Friedman and Neale 1992; Miller et al. 1989; Wheeler and Jech 1992). Isozymes generally provide ample genetic information and are relatively inexpensive, rapid, and technically easy to apply, thus they should continue to play an important role in forest tree improvement. In recent years, DNA-based genetic markers have been developed, most notably restriction fragment length polymorphisms (RFLPs) and polymerase chain reaction- (PCR) based markers. DNA-

based markers have the potential to overcome some of the limitations of isozymes for tree improvement applications. In this paper, we will describe some of the new DNA-based genetic markers and attempt to show where they might best be applied in forest tree improvement. The application of DNA markers in forest genetics research in general has been discussed in several recent papers (Cheliak and Rogers 1990; Friedman and Neale 1992; Gianfranceschi et al. 1991; Nance and Nelson 1989; Neale et al. 1989; Neale and Williams 1991; Wagner 1992).

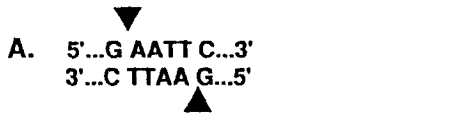
DNA-based genetic markers

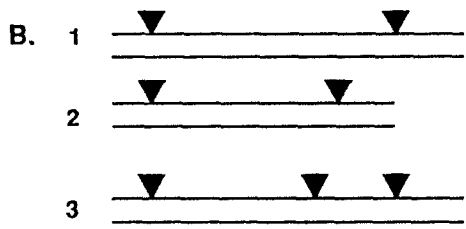
Genetic markers are of two general types; Mendelian and nonMendelian. Mendelian markers segregate as alleles at a locus, whereas allelism is not established for nonMendelian markers. NonMendelian markers are often called fingerprints. There are also two major assay approaches, RFLPs and PCR, both of which are used with Mendelian and nonMendelian markers. We will describe markers of both types and assay approaches.

Restriction fragment length polymorphisms

The concept of RFLP mapping of complex genomes was first described by Botstein et al. (1980) and has been described by many authors (Beckmann and Soller 1983, 1986a, 1986b; Landry and Michelmore 1987; Soller and Beckmann 1983; Tanksley et al. 1989). RFLPs are simple Mendelian genetic markers which result from various types of mutations and rearrangements of the DNA. The first step in RFLP detection is to isolate DNA from the organism of study and cleave the DNA with one or more restriction endonucleases. Restriction endonucleases are bacterial enzymes which cleave double-stranded DNA at unique palindromic recognition sequences, usually 4—8 nucleotides in length. The restriction endonuclease *Eco*RI, for example, cleaves the 6-base sequence GAATTC between the G and A (Fig. 1A). Insertions and deletions of small segments of DNA or the gain or loss of a restriction site are two types of RFLPs which are easily detected (Figs. 1B and 1C). Eukaryotic genomes, however, are very large so that when genomic DNA is cut with a restriction enzyme, a nearly continuous distribution of restriction fragment sizes results. It is thus impossible to visualize individual DNA fragments as shown in Fig. 1C.

The problem of visualizing individual DNA fragments is overcome by a technique called Southern blotting and probe hybridization. Following digestion with restriction enzymes, DNAs are fractionated electrophoreti-





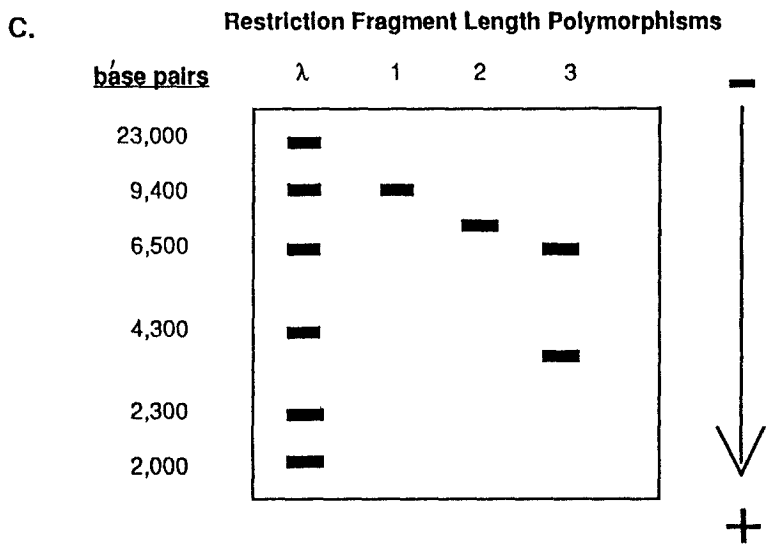


Fig. 1. Restriction fragment length polymorphisms. A. The recognition sequence for the restriction endonuclease *Eco*RI is 5'..GAATTC..3'. *Eco*RI cuts double-stranded DNA between the G and A within the palindromic 6-base sequence. B. DNA fragments of three trees cut with *Eco*RI. Tree 2 has a deletion of a small amount of DNA sequence between the two *Eco*RI sites, whereas tree 3 has gained an *Eco*RI site relative to trees 1 and 2. C. Gel separation of *Eco*RI-cut DNA fragments. Lanes 1, 2, and 3 show the separation of DNA fragments from the three trees. Molecular weight standards are shown in the first lane.

cally on agarose gels. The DNA is then denatured and single-stranded DNA molecules are transferred and covalently linked to nylon membranes (blots). Small DNA fragments, called probes, are then radiolabelled and allowed to hybridize to their complementary DNA sequences bound to the blots. The radioactive DNA hybrids on the blot are visualized by autoradiography (Fig. 2).

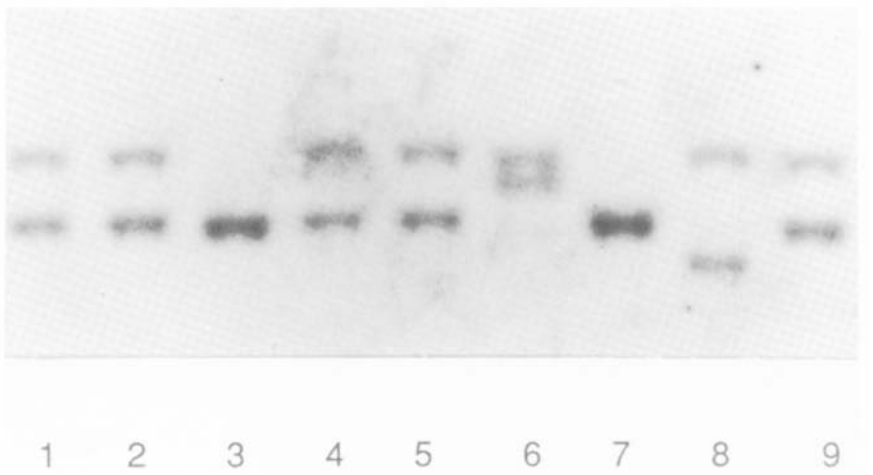


Fig. 2. Autoradiogram showing RFLPs among nine Douglas-fir seed orchard clones cut with *Eco*RI and hybridized with a cDNA probe from Douglas-fir.

Polymerase chain reaction-based markers

Amplification of specific DNA sequences by the polymerase chain reaction (Sakai et al. 1988) offers a new and powerful approach to developing genetic markers. Several approaches have recently been developed, however the most widely used method is the random amplified polymorphic DNA (RAPD) marker (Williams et al. 1990). Short oligonucleotide primers are used for PCR and resulting amplification products appear as visible bands on gels. If complementary sequences to the primer do not exist then no amplification product or visible band on gels results. Thus, these are dominant genetic markers because the homozygote for the amplification product can not be distinguished from the heterozygote. In conifers, however, the problem of dominance can be overcome if RAPD markers are assayed from the haploid megagametophyte tissue.

Fingerprinting markers

A common use of genetic markers is to establish whether two or more individuals are genetically the same or different. This application is often called "fingerprinting" from the traditional method of human forensic science. Genetic markers used for fingerprinting often reveal a large number of complex polymorphisms and are generally nonMendelian markers. The first well characterized DNA fingerprinting probes were the human minisatellite probes (Jefferies et al. 1985). Two of these probes, clones 33.15 and 33.6, have been shown to cross hybridize and reveal polymorphisms in rice (Dallas 1988) and in *Populus* (Rogstad et al. 1988). However, the lack of reports on the use of these probes with other plants suggests that their utility in plants is limited.

Another DNA fingerprinting probe is the bacteriophage M13 subrepeat probe. Vassart et al. (1987) showed that M13 would cross hybridize to mammalian minisatellite sequences and subsequently it was demonstrated that it would hybridize to plant DNA as well (Ryskov et al. 1988; Rogstad et al. 1988). Nybom and coworkers have used the M13 probe to fingerprint varieties of species with the Rosaceae (Nybom 1990; Nybom and Schaal 1990a, 1990b; Nybom et al. 1989, 1990). Rogstad et al. (1988) showed that M13 would cross hybridize to *Picea glauca, Pinus torreyana*, and *Populus* species and that polymorphisms could be detected. These preliminary studies, however, have not been followed by larger studies to evaluate the efficacy of using M13 to fingerprint in large populations of forest trees.

A PCR-based approach to revealing M13 polymorphisms has recently been developed (Welsh and McClelland 1990). The M13 DNA sequencing primers are used for PCR amplification of genomic DNA. This technique, called arbitrarily primed (AP-PCR), was used to fingerprint three varieties of rice (Welsh and McClelland 1990).

Finally, a new and promising marker called simple sequence length polymorphism (SSLP) might be used for fingerprinting in plants as well as for mapping individual genetic loci (Litt and Luty 1989; Tautz 1989; Weber and May 1989). Eukaryotic genomes contain stretches of simple sequences of tandem repititions of mono-, di-, tri-, and tetrameric motifs. Because these simple sequences vary in length and are dispersed throughout the genome, they are potentially useful markers for fingerprinting. Weising et al. (1991) have shown that such sequences exist in plants and can be used for fingerprinting. In addition, it may also be possible to design PCR primers from the unique sequence regions flanking the simple repeat sequences to reveal individual genetic loci (Tautz 1989). This

approach is being used to map CA- repeat regions in humans (Litt and Luty 1989).

Advantages of molecular markers

DNA markers are true genetic markers which have several distinct advantages over other biochemical markers for some common applications in tree improvement. The first, and probably most important, advantage is that potentially an unlimited number of DNA markers can be detected. The number of biochemical markers, however, is limited to a small number of isozyme and monoterpene markers. A second advantage is that DNA markers do not vary among tissue types or developmental stages of the plant because the assays are based on the DNA itself and not the products of genes. There are clearly differences in the levels of expression of certain isozymes among tissue types commonly used in isozyme assays (megagametophytes, embryos, buds, needles). In Douglas-fir, there are only 11 isozyme loci which can be assayed from all four of the tissues listed above (Adams et al. 1990). DNA, however, can be isolated from almost any plant part, thus enabling DNA marker detection from many tissue types and at most developmental stages. A third advantage of DNA markers is that they are not affected by environmental variation. The presence or abundance of isozyme or biochemical marker products can be affected by environmental stimuli. A good example is the enzyme alcohol dehydrogenase (ADH). There are two isozyme loci on gels stained for ADH; however, one of the loci is anaerobically induced and is undetectable in noninduced tissue (Harry et al. 1988). Using DNA technology, both loci would be detected, whether the tissue were subjected to anaerobosis or not. There may also be allelic variation in response to environmental stimuli at certain isozyme loci.

RFLP and RAPD markers have some important advantages and disadvantages relative to one another. RAPD markers are generally simpler and faster to assay for than RFLPs. They require as little as 10ng of DNA per assay as opposed to RFLPs which require 10ng or more in conifers. PCR assays also do not require the use of radioisotopes which are used with RFLPs. The most severe limitation of RAPD markers is that they are dominant markers. Dominant markers are less informative than codominant markers for many of the applications in forest tree improvement. In addition, RAPD loci are diallelic; all that can be detected at a locus is the presence or absence of a PCR product. RFLPs, however, can detect multiple alleles at a locus. A PCR-based codominant genetic marker which would reveal multiple alleles would be an important development for genetic studies in forest trees and other plants.

Applications of molecular markers in tree improvement

Molecular markers can be used to monitor the success of numerous tree improvement activities. The application of isozyme markers to such problems has been well documented (Adams 1981a, 1983; Adams et al. 1988; Wheeler and Jech 1992). In this section, we will identify areas where DNA-based markers might be more powerful but also identify areas where isozymes would still be the marker of choice.

Estimation of genetic variability in domesticated populations

Isozymes have been used to assess the amounts of genetic variability in selected breeding populations versus wild stands from where selections were made, in seed orchard crops, and in commercial seed collections from wild stands (Adams 1981b). In Douglas-fir, these data do not suggest large losses in genetic variability due to domestication (Adams 1981b). In most cases, estimates from isozymes can be obtained rapidly and inexpensively, and a large number of loci can be assayed if megagametophytes are used. Thus, for rapid assessments of variability in domesticated populations and outputs from such populations (e.g., seed crops), isozymes are probably the best approach. However, in species in which levels of isozyme variability are low or in which estimates must be obtained from tissues where only a few loci can be resolved (e.g., seedlings in nursery crops), the application of DNA-based markers would be warranted. RFLPs would be a better marker than RAPDs for this application because multiple alleles at a locus can be detected.

Germplasm identification

Probably the most important application of genetic markers in tree improvement is the broad problem of germplasm identification. The array of identification problems ranges from simple to complex. For example, a simple identification problem would be to determine if two ramets are members of the same or different clone. This problem is akin to those in human forensics. Any reproducible genetic difference between the two ramets would be considered sufficient evidence to conclude that they were members of different clones. A more complex problem would be to assign seed source identification to a bulked seed collection. A problem of this type would generally involve gene frequency estimation. Whether a particular problem is simple or complex, the power to make an accurate identification is a function of genetic discrimination.

The power to genetically discriminate between individuals or groups of

individuals is a function of the number of markers and the amount of genetic variability revealed by the markers. Forest trees, especially conifers, have high levels of isozyme variation, which makes these markers quite adequate for many germplasm identification problems. One limitation of isozymes is that a large number of loci can not be resolved from some tissues. This limitation could be overcome with DNA markers. RFLPs would be useful where large (> 5g) amounts of tissue are available to extract the large amount of DNA needed. RAPD markers would be useful for some types of identification, but will have limited value because of dominance. The most important type of marker for germplasm identification are the fingerprinting markers such as M13, AP-PCR, SSLPs, or other hypervariable sequences. We recently used high-copy RFLP probes from loblolly pine to fingerprint 12 unlabelled longleaf pine seed orchard clones which we were unable to genetically differentiate using isozymes.

Controlled crosses

Controlled crossing is an important aspect of most tree breeding programs. Adams et al. (1988) have used isozymes to determine the accuracy of such crosses from operational programs. Their results showed that 30% or more of the Douglas-fir and loblolly pine crosses they studied were not correct and that these determinations could be made with as few as 6—10 isozyme loci and five seeds per cross. For these species, and possibly other forest trees, it would be unnecessary to use DNA markers. RFLPs would be useful for species with low isozyme variation, however, it would be necessary to grow the progeny to a size large enough to extract the large amount of DNA needed. RAPDs will be of little value for this application because of dominance.

Seed orchards

There are numerous applications of genetic markers in seed orchard management (Wheeler and Jech 1992). They include: (1) estimation of the extent of pollen contamination (Friedman and Adams 1985; Smith and Adams 1983), (2) estimating selfing rates and inbreeding (El-Kassaby et al. 1986), (3) estimating the success of supplemental mass pollination (Joly and Adams 1983; Wheeler and Jech 1986), (4) determining mating patterns within orchards (Erickson and Adams 1989), and (5) determining the effects of cultural practices, such as top pruning (Omi and Adams 1985). These applications are all centered around determining male parentage of seed produced in the orchard. By using isozymes, the geno-

type of the pollen gamete is inferred from the genotypes of the seed megagametophyte and embryo and then compared to male genotypes within and outside the orchard to infer parentage. The power of genetic discrimination among putative males is a function of the number of genetic markers, the amount of allelic variability at individual loci, the frequencies of alleles in the population, and the overall number of males in the population. Complete genetic discrimination among males by using isozymes is never possible in operational seed orchard situations and therefore statistical methods of inference must be employed. DNA markers could potentially increase genetic discrimination, but unfortunately, neither RFLPs or RAPDs are well suited for inferring the genotype of the male gamete from the embryo. A codominant PCR-based marker would be extremely useful for this purpose.

Quantitative trait mapping and marker-assisted selection

The uses of molecular and biochemical markers discussed previously do not require a full understanding of the linkage relationships among the markers. Isozyme linkage analyses have been conducted for numerous tree species (e.g., Adams and Joly 1980; Conkle 1981; Guries et al. 1978), however, the number of loci included in these studies was small, and the number of linkage blocks was generally less than the number of chromosomes. Such maps are useful for showing linkage relationships among isozyme loci, but restrict investigation to small segments of the genome. This limitation can be overcome by DNA markers because there is potentially an unlimited number that can be mapped. Once a saturated genetic map has been constructed it then becomes theoretically possible to map individual loci coding for quantitative traits (QTLs). After associations between markers and QTLs have been established it may be possible to use this information predictively in a marker-assisted selection scheme.

Quantitative trait locus mapping

The first demonstration of quantitative trait mapping using a saturated molecular marker map was in tomato (Paterson et al. 1988). Loci for three traits (fruit mass, soluble solids, and pH) were mapped in a backcross population of 237 plants derived from an interspecific cross. A subset of 68 (63 RFLP and 5 isozyme) evenly distributed markers were selected from a saturated map of over 300 markers. Interval mapping

(Lander and Botstein 1989) was used to identify chromosomal regions showing strong associations with quantitative traits. Each region includes one or more quantitative trait loci. Modifications of this approach could be applied to detecting QTLs of important traits in trees.

Stem volume is probably the most commercially important quantitative trait in forest trees. This trait is controlled by many genes and has a low heritability. Polygenic control and low heritability would make it difficult to map QTLs for stem volume. Nevertheless, if a small subset of genes having large effects could be detected, their identification would have large economic importance. Mapping of more highly heritable traits, controlled by a small number of genes (e.g., wood specific gravity, resistances to diseases, and phenological traits), would be easier and might be better choices for initial investigations.

Quantitative trait mapping in agricultural crops has been conducted using F_2 or backcross pedigrees. Such pedigrees generally do not exist for forest trees and are difficult to construct. QTL mapping in trees would have to be conducted using multigeneration outbred pedigrees. Unfortunately, the statistical theory for QTL mapping from outbred tree pedigrees is generally lacking. It is likely, however, that the interval mapping approach of Lander and Botstein (1989) can be extended to multigeneration outbred pedigrees.

Marker-assisted selection

Early genetic selection in forest tree breeding would have enormous practical importance because of the long generation times in trees. Early selection on height growth has been successfully practiced on seedlings at age two and older (Magnussen and Yeatman 1987; McKinley and Lowe 1986; Williams 1987) but has not been successful with one-year-old seedlings (Waxler and van Buijtenen 1981; Williams 1986). Indirect selection based on DNA markers could be practiced at a very early age if linkages to important traits are found. In addition, costs would be lower than progeny testing, selection intensities might be much higher, and selection may potentially be more efficient because of the higher heritabilities of the markers.

One cannot assume, however, that marker-assisted selection in forest trees can be practiced as effectively as it may be with highly inbred agricultural crops. Forest tree breeding populations are very large and are nearly in linkage equilibrium. Linkage equilibrium makes it difficult to achieve much genetic gain based on marker selection. If small elite breeding populations are employed, then it would be possible to practice within

family selection, based on the markers, if the phases of parent tree marker genotypes were known. Further advancements in DNA mapping techniques and new statistical methods might enable marker-assisted selection under less restricted conditions. Forest geneticists should prepare for this possibility by developing high-density molecular maps and testing the efficiency of marker-assisted selection in experimental populations.

Mapping of DNA markers in conifers

We are constructing genetic maps in conifers using DNA markers. In this section, we will briefly describe the approaches we are using to construct these maps. Our initial project was to construct an RFLP map for loblolly pine (*Pinus taeda* L.) based on a segregating F_2 population of a 3-generation outbred pedigree. DNA is isolated from each of the four grandparents and two parent trees of the pedigree (Fig. 3). DNAs are digested with the restriction enzyme *Hin*dIII and Southern blots are prepared (Devey et al. 1991). Blots are then hybridized with ^{32}P-labelled random cDNA (complementary DNA) or genomic DNA probes (Fig. 4). Segregating RFLPs in this cross are identified from these hybridizations. We limit our screening for polymorphisms to just *Hin*dIII because nearly all

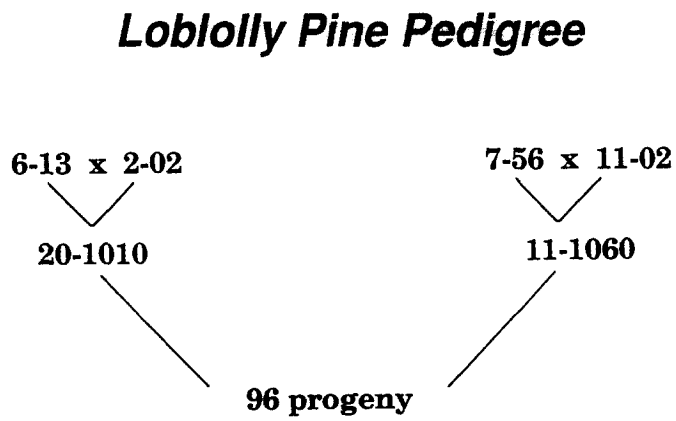


Fig. 3. Three-generation pedigree from loblolly pine to be used in construction of a high-density RFLP map.

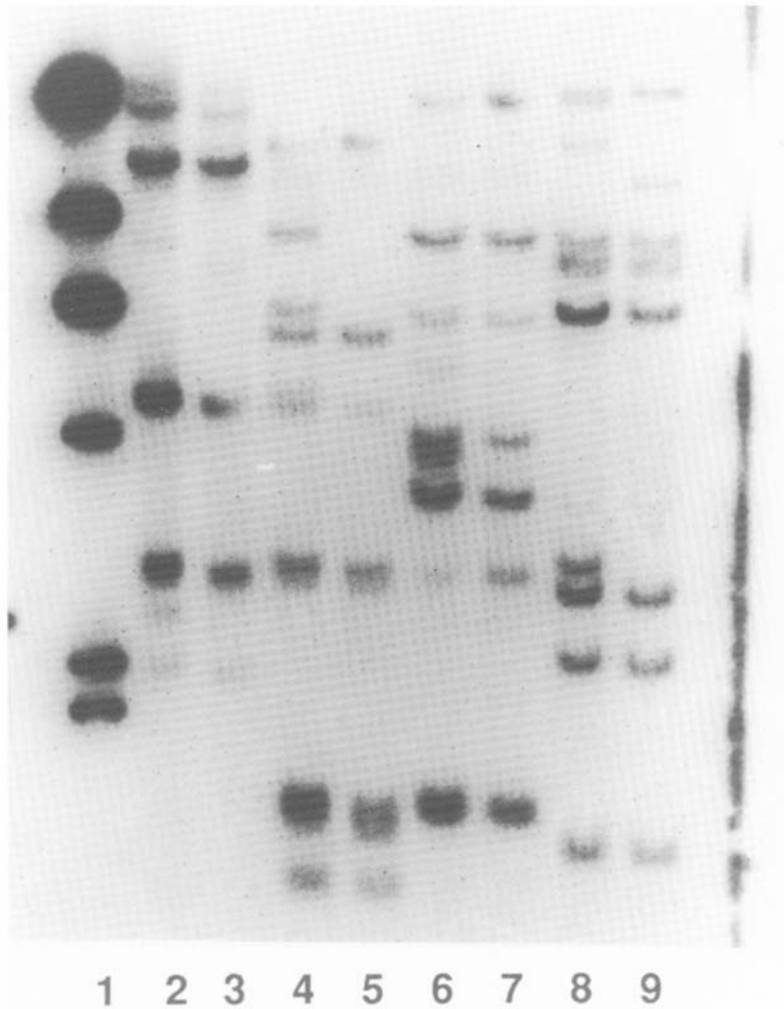


Fig. 4. Autoradiogram of two loblolly pine parent tree DNAs, 20-1010 and 11-1060, cut with four restriction enzymes and hybridized with loblolly pine cDNA probe pPtIFGc653. Lane 1, lambda*Hind*III molecular weight marker. Lanes 2—3, *Bam*HI; lanes 4—5, *Dra*I; lanes 6—7, *Eco*RI; and lanes 8—9, *Hin*dIII.

polymorphisms are length variants; other enzymes rarely reveal additional variation (Devey et al. 1991). Once a segregating RFLP is identified, the probe is relabelled and hybridized to a set of blots containing the 96 progeny DNA (Fig. 5). Segregating alleles are scored and tests for linkage

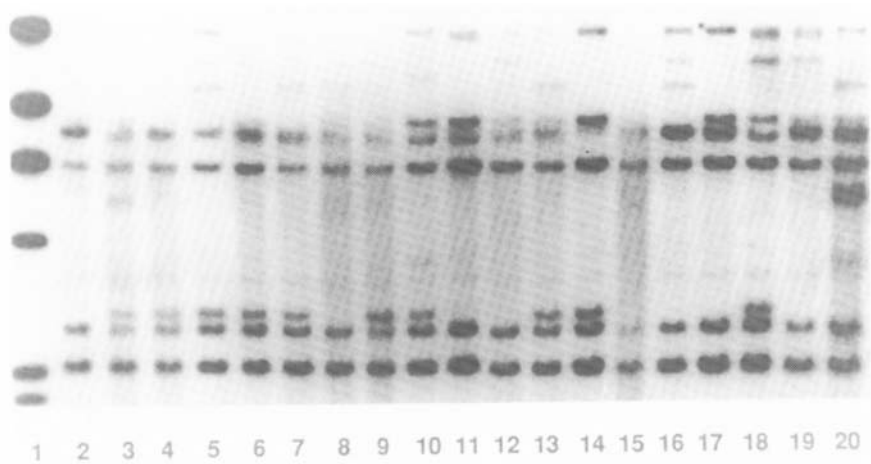


Fig. 5. Autoradiogram of segregating progeny of loblolly pine cross 20-1010 X 11-1060 cut with *Hin*dIII and hybridized with cDNA probe pPtIFGc653 (lanes 2-2-). Lane 1, lambda*Hin*dIII molecular weight marker.

are performed. We are following a nearly identical strategy for mapping RFLPs in a 3-generation Douglas-fir pedigree.

We have also begun to add RAPD markers to our loblolly pine and Douglas-fir RFLP maps. Segregating RAPDs in the pedigree are identified by screening random 10-mers (Operon Technologies) against DNA from four megagametophytes from both parent trees. Primers for segregating RAPDs are then used with DNAs from the F_2 progeny. RAPD markers can also be easily mapped using segregating megagametophytes from heterozygous mother trees.

Acknowledgments

We thank Bob Weir and Steve McKeand of North Carolina State University and Claire Williams and Nick Wheeler of the Weyerhaeuser Company for providing pedigrees and Dave Harry and Yousry El-Kassaby for reviews of the manuscript. This project was partially supported by U.S.D.A. Forest Biology Grant No. 88-33520-4074.

References

Adams, W. T. 1981a. Applying isozyme analyses in tree-breeding programs. pp. 60—64. In: Conkle, M. T. (Technical Coordinator) Proc. Symp. Isozymes North Am. Forest Trees and Forest Insects. USDA Forest Serv., Pacific SW Forest and Range Exp. Sta., Berkeley, California, Gen. Tech. Rep. PSW-48.

Adams, W. T. 1981b. Population genetics and gene conservation in pacific northwest conifers. pp. 401—415. Scudder, G. G. E. and Reveal, J. L. (Eds), Evolution Today, Proceedings of the Second International Congress of Systematic and Evolutionary Biology.

Adams, W. T. 1983. Application of isozymes in tree breeding. pp. 381—400. In: Tanksley, S. D. and Orton, T. J. (Eds), Isozymes in Plant Genetics and Breeding, Part A. Elsevier Science Publishers B. V. Amsterdam.

Adams, W. T. and Joly, R. J. 1980. Genetics of allozyme variants in loblolly pine. J. Hered. 71: 33—40.

Adams, W. T., Neale, D. B., Doerksen, A. H. and Smith, D. B. 1990. Inheritance and linkage of isozyme variants from seed and vegetative bud tissues in coastal Douglas-fir [*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco]. Silvae Genet. 39: 153—167.

Adams, W. T., Neale, D. B. and Loopstra, C. A. 1988. Verifying controlled crosses in conifer tree-improvement programs. Silvae Genet. 37: 147—152.

Beckmann, J. S., and Soller, M. 1983. Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and costs. Theor. Appl. Genet. 67: 35—43.

Beckmann, J. S., and Soller, M. 1986a. Restriction fragment length polymorphisms and genetic improvement of agricultural species. Euphytica 35: 111—124.

Beckmann, J. S., and Soller, M. 1986b. Restriction fragment length polymorphisms in plant genetic improvement. Oxford Surv. Plant Mol. Cell Biol. 3: 196—249.

Botstein, D., White, R. L., Skolnick, M., and Davis, R. W. 1980. Construction of genetic linkage map in man using restiction fragment length polymorphisms. Am. J. Hum. Genet. 32: 641—656.

Cheliak, W. M. and Rogers, D. L. 1990. Integrating biotechnology into tree improvement programs. Can. J. For. Res. 20: 452—463.

Cheliak, W. M., Yeh, F. C. H. and Pitel, J. A. 1987. Use of electrophoresis in tree improvement programs. For. Chron. 63: 89—96.

Conkle, M. T. 1981. Isozyme variation and linkage in six conifer species. pp. 11—17. In: Conkle, M. T. (Technical Coordinator), Proc. Symp. Isozymes North Am. Forest Trees and Forest Insects. USDA Forest Serv., Pacific SW Forest and Range Exp. Sta., Berkeley, California, Gen. Tech. Rep. PSW-48.

Dallas, John F. 1988. Detection of DNA "fingerprints" of cultivated rice by hybridization with a human minisatellite DNA probe. Proc. Natl. Acad. Sci. USA. 85: 6831—6835.

Devey, M. E., Jermstad, K. D., Tauer, C. G. and Neale, D. B. 1991. Inheritance of RFLP loci in a loblolly pine three-generation pedigree. Theor. Appl. Genet. 83: 238—242.

El-Kassaby, Y. A., Yeh, F. C. and Sziklai, O. 1986. Clonal and seedling seed orchards: A comparison of outcrossing rates and heterozygosity in coastal Douglas-fir using allozyme markers. pp. 410—421. In: Hatcher, A. V. and Weir, R. J. (Eds). IUFRO Conference Proc., A Joint Meeting of Working Parties-S2.04.02, S2.04.03., and S2.03.03. Williamsburg, Virginia.

Erickson, V. J. and Adams, W. T. 1989. Mating success in a coastal Douglas-fir seed orchard as affected by distance and floral phenology. Can. J. For. Res. 19: 1248—1255.

Friedman, S. T. and Adams, W. T. 1985. Estimation of gene flow into two seed orchards of loblolly pine (*Pinus taeda* L.). Theoret. Appl. Genet. 69: 609—615.

Friedman, S. T. and Neale, D. B. 1992. Biochemical and molecular markers. In: Bramlett, D. (Ed), Pollen Management Handbook, Vol. II. SE Exp. Sta., Ashville, North Carolina, in press.

Gianfranceschi, L., Taramino, G., Binelli, G., Pe, M. E. and Ottaviano, E. 1991. RFLP markers in the genetic analysis of forest trees. In Effects of Pollution on the Structure of Forest Tree Populations, Consiglio Nazionale delle Ricerche, pp. 67—78.

Guries R. P., Friedman, S. T. and Ledig, F. T. 1978. A megagametophyte analysis of genetic linkage in pitch pine (*Pinus rigida* Mill.). Heredity 40: 309—314.

Harry, D. H., Kinlaw, C. S. and Serderoff, R. R. 1988. The anaerobic stress response and its use for studying gene expression in conifers. pp. 275—290. In: Hanover, J. W. and Keathely, D. E. (Eds), Genetic Manipulation of Woody Plants. Plenum Publishing Corporation, New York.

Jeffreys, A. J., Wilson, V. and Thein, S. L. 1985. Individual-specific "fingerprints" of human DNA. Nature 316: 76—79.

Joly, R. J. and Adams, W. T. 1983. Allozyme analysis of pitch x loblolly pine hybrids produced by supplemental mass-pollination. Forest Sci. 29: 423—432.

Lander, E. S. and Botstein, D. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185—199.

Landry, B. S. and Michelmore, R. W. 1987. Methods and applications of restriction fragment length polymorphism analysis to plants. In: Tailoring genes for crop improvement: an agricultural perspective. Ed. by: G. Bruening, J. Harada, and A. Hollaender. Plenum Press, New York. pp. 25—44.

Litt, M. and Luty, J. A. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am. J. Hum. Genet. 44: 397—401.

Magnussen, S. and Yeatman, C. W. 1987. Four years' height growth of 25 jack pine (*Pinus banksiana* Lamb.) families in an accelerated nursery trial. pp. 47—64. In: Proc. 30th Northeastern For. Tree Improv. Conf., Orono, Maine.

McKinley, C. R. and Lowe, W. J. 1986. Juvenile-mature correlations. pp. 11-16. In: Proc. Workshop on Advanced Generation Breeding: Current Status and Research Needs. Baton Rouge, Louisiana. South. Coop. Series Bull. No. 309.

Miller, R. G., Conkle, M. T. and Friedman, S. T. 1989. The Forest Service laboratory for genetic analyses of trees. Three Planters' Notes 40: 25—29.

Nance, W. L. and Nelson, C. D. 1989. Restriction fragment length polymorphisms and their use in marker assisted selection in Southern pine improvement. pp. 50—59. In: Proceedings 20th Southern Forest Tree Improvement Conference. Charleston, South Carolina. The National Technical Information Service, Springfield, Virginia.

Neale, D. B., Tauer, C. G., Gorzo, D. M., and Jermstad, K. D. 1989. Restriction fragment length polymorphism mapping of loblolly pine: Methods, applications, and limitations. pp. 363—372. In: Proc. 20th Southern Forest Tree Imp. Conf., Charleston, South Carolina. The National Technical Information Service, Springfield, Virginia.

Neale, D. B. and Williams, C. G. 1991. Restriction fragment length polymorphism mapping in conifers and applications to forest genetics and tree improvement. Can. J. For. Res. 21: 545—554.

Nybom, H., Schaal, B. A. and Rogstad, S. H. 1989. DNA "fingerprints" can distinguish cultivars of blackberries and raspberries. Acta Horticulturae 262: 305—310.

Nybom, H. 1990. Genetic variation in ornamental apple trees and their seedlings (Malus, Rosaceae) revealed by DNA "fingerprinting" with the M13 repeat probe. Hereditas 113: 17—28.

Nybom, H. and Schaal, B. A. 1990a. DNA "fingerprints" applied to paternity analysis in apples (Malus x domestica). Theor. Appl. Genet. 79: 763—768.

Nybom, H. and Schaal, B. A. 1990b. DNA "fingerprints" reveal genotypic distributions in natural populations of blackberries and raspberries (rubus, rosaceae). Amer. J. Bot. 77: 883—888.

Nybom, H., Rogstad, S. H. and Schaal, B. A. 1990. Genetic variation detected by use of the M13 "DNA fingerprint" probe in Malus, Prunus, and Rubus (Rosaceae). Theor. Appl. Genet. 79: 153—156.

Omi, S. K. and Adams, W. T. 1985. Variation in seed set and proportions of outcrossed progeny with clones, crown position, and top pruning in a Douglas-fir seed orchard. Can. J. For. Res. 16: 502—507.

Paterson A. H., Lander, E. S., Hewitt, J. D., Paterson, S., Lincoln, S. E. and Tanksley, S. D. 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335: 721—726.

Rogstad, S. H., Patton, J. C. and Schaal, B. A. 1988a. A human minisatellite probe reveals RFLPs among individuals of two angiosperms. Nucl. Acids Res. 16: 11378.

Rogstad, S. H., Patton, J. C. and Schaal, B. A. 1988b. M13 repeat probe detects DNA minisatellite-like sequences in gymnosperms and angiosperms. Proc. Natl. Aca. Sci. USA, 85: 9176—9178.

Ryskov, A. P., Jincharadze, A. G., Prosnyak, M. I., Ivanov, P. L. and Limborska, S. A. 1988. M13 phage DNA as a universal marker for DNA finger printing of animals, plants and microorganisms. FEBS LTRS 233: 388—392.

Sakai, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. and Arnheim, N. 1985. Enzymatic amplification of B-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230: 1350—1354.

Smith, D. B. and Adams, W. T. 1983. Measuring pollen contamination in clonal seed orchards with the aid of genetic markers. pp. 69—77. In: Proc. 17th South. Conf. on Forest Tree Improv.

Soller, M. and Beckmann, J. S. 1983. Genetic polymorphism in varietal identification and genetic improvement. Theor. Appl. Genet. 67: 25—33.

Tanksley, S. D., Young, N. D., Paterson, A. H. and Bonierbale, M. W. 1989. RFLP mapping in plant breeding: new tools for an old science. BioTechnology, 7: 257—264.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucl. Acids Res. 17: 6463—6471.

Vassart, G., Georges, M., Monsieur, R., Brocos, H., Geguaere, A. S. and Christophe, D. 1987. A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. Science 235: 683—684.

Wagner, D. B. 1992. Nuclear, chloroplast, and mitochondrial DNA polymorphisms as biochemical markers in population genetic analyses of forest trees. New Forests 6: 373—390.

Waxler, M. S. and van Buijtenen, J. P. 1981. Early genetic evaluation of loblolly pine. Can. J. For. Res. 11: 351—355.

Weber, J. L. and May, P. E. 1991. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am. J. Hum. Genet. 44: 388—396.

Weising, K., Beyermann, B., Ramser, J. and Kahl, G. 1991. Plant DNA fingerprinting with radioactive and digoxigenated oligonucleotide probes complementary to simple repetitive DNA sequences. Electrophoresis 12: 159—169.

Welsh, J. and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acids Res. 18: 7213—7218.

Wheeler, N. C. and Jech, K. S. 1986. Estimating supplemental mass pollination (SMP) success electrophoretically. pp. 111—120. In: Proceedings Canadian Tree Improvement Association. Quebec City, Canada.

Wheeler, N. C. and Jech, K. S. 1992. The use of electrophoretic markers in seed orchard research. New Forests 6: 311—328.

Williams, C. G. 1986. Early genetic testing for loblolly pine growth traits. Ph. D. dissertation, North Carolina State University, Raleigh.

Williams, C. G. 1987. Influence of shoot ontogeny on juvenile-mature correlations in loblolly pine. For. Sci. 33: 411—422.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18: 6531—6535.